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Review Article

Comparative Evaluation of Antimicrobial Efficacy of Aloe Barbadensis Miller, Curcuma Longa and Azadirachta Indica with Sodium Hypochlorite: An Invitro Study

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Abstract:

Context: The thorough removal of microflora, debris, and irritants from the root canal system is necessary for root canal treatment to be effective. Herbal or natural products are becoming more popular recently as a result of their strong antibacterial activity, biocompatibility, anti-inflammatory, and anti-oxidant properties

Aims: To compare and evaluate antimicrobial efficacy of herbal extracts Aloe barbadensis miller, Curcuma longa and Azadirachta indica with 2.5% sodium hypochlorite against the Enterococcus faecalis .

Settings and Design: Forty muller Hinton agar plates were taken as sample and were grouped into 4 groups as Group I: 2.5% NaOCl ,Group II: Curcuma longa extract ,Group III: Azadirachta indica extract, Group IV: Aloe barbadensis miller.

Methods and Material: The agar plates were inoculated with strains of E.faecalis (ATCC29212) and test solutions were placed in agar wells in group 1-4. The agar plates were incubated at 37 ^{0}C for 72 hours. After incubating, the agar plates were examined for zone of inhibition for assessing the degree of susceptible or resistance of test organism.

Statistical analysis used: Statistical analysis was performed using Oneway Anova test with statistical significance at $P < 0.05$.

Results: It was shown that Curcuma longa was highly efficient similar to 2.5%NaOCl

Conclusions: Curcuma longa extract has a significant antimicrobial efficacy against *Enterococcus faecalis* similar to 2.5% sodium hypochlorite
Key-words: Endodontic irrigant, *Enterococcus faecalis*, Herbal extracts, Microbiology.
Key Messages: Herbal irrigants are best alternative to gold standard chemical endodontic irrigants. With their increase in antimicrobial properties, they aid in complete removal of endodontic microorganisms.

Introduction

The primary objective of endodontic treatment relies on the elimination of microorganisms from the root canal system[1]. Numerous irritants persist within the complex root canal system due to pathological changes in the dental pulp. The progress of these irritants from the infected canals into the surrounding tissues initiates the formation and perpetuation of peri-radicular lesions and this response is manifested as an immune inflammatory reaction[2]. *E.faecalis*, a gram positive and facultative anaerobe is the most prevalent bacteria found in persistent and secondary infections and its prevalence ranges from 24-77% which is responsible for 80–90% of enterococcal infection[3]. This frequent presence of Enterococcus faecalis in root canals where endodontic treatment has failed suggests that, this is an opportunist pathogen, whose persistence in the canals represents a significant therapeutic problem[4].

Currently, the gold standard irrigating solutions are sodium hypochlorite and chlorhexidine for canal irrigation. However, constant increase in antibiotic resistance and side effects of chemical irrigants has steered to the search for alternative herbal medicaments. Herbal extracts with their superior properties like ease of availability, cost effectiveness, low toxicity, anti-bacterial and anti-inflammatory effects can be a potential alternative. Various herbal extracts, such as Aloe barbadensis miller, Azadirachta indica, *Morinda citrifolia*, and Curcuma longa are having, anti-inflammatory, anti-microbial and therapeutic effects[5]. These are promising organic/natural products that can be used as endodontic irrigants.

Thus the aim of our study is to assess and evaluate the antibacterial effects and insilico docking of Aloe barbadensis miller, curcuma longa and Azadirachta indica herbal extracts that can be employed as root canal irrigating agents with 2.5% sodium hypochlorite.

Subjects and Methods:

This study was approved by the institutional ethics committee of Tagore Dental College [IEC/TDCH/99/2021]

Culture of *E.Faecalis*:

Standard strain of Enterococcus *faecalis* (ATCC 29212) (HiMedia, Mumbai) spores are grown and maintained in 25 mL of Brain-Heart Infusion (BHI) broth (HiMedia Laboratories, Mumbai) by incubating at 37^{o}C for 24 hours. Viable bacterial growth was indicated by a change in turbidity of the solution. The broth culture suspension of bacteria was adjusted at a turbidity equivalent to the barium sulfate standard of 0.5 McFarland units (equivalent to $1.5x10^{8}$ CFU/ml), with sterile BHI taken as standard.

Agar Diffusion Test:

Forty Mueller Hinton Agar (MHA) was selected as medium for the growth of E. faecalis. A sterile cotton swab is dipped into the BHI bacterial suspension, rotated on the side of the tube to remove surplus and used to inoculate the agar plates. All the plates are uniformly inoculated by streaking evenly in three directions. (Research Centre, Tagore Medical College and Hospital). After the inoculum is dry, a sterile 6 mm cork borer is used to prepare 4 equally spaced wells were bored in the agar plate.

Preparation of extracts

Preparation of Curcuma longa extract: Curcuma longa rhizomes were washed with distilled water and patted dry. They were cut in pieces and completely dried in an oven by a tray-drying process at a temperature of 40± 5 °C for a period of about 7-10 days till they were moisture-free. The cut pieces were grounded to coarse powder. This was placed in a large glass chamber into which 80 mL of sterile distilled water was added to prepare the aqueous extract. The glass chamber was closed with a glass lid to prevent evaporation of the menstruum and the chamber was allowed to stand for seven days with occasional stirring of the contents. The liquid was then strained and the solid residue, called "marc", was pressed to recover as much solution as possible and clarified by filtration. Pure turmeric extract was taken and mixed with 80 mL distilled water.

Preparation of Azadirachta indica extract: Mature fresh Azadirachta indica leaves were collected, and taxonomic identification of the plant was performed. In a beaker with 800 mL of distilled water, neem leaves weighing 100 g were tied in a muslin cloth and heated over a low flame. The extract was reduced to 400 ml to obtain 25% concentration of aqueous neem extract. cooling, the extract was filtered using a filter paper and collected for usage.

***Preparation of A. vera extract*:** Leaves of *Aloe vera* were collected. Fresh 100 g of A. vera leaves were processed into a liquid by removing the pulp using a mixer. 5 parts of distilled water were combined with 1 part of A.vera. The mix was dehydrated in a water bath to obtain the extract which was dissolved in methanol for further use as an irrigating agent.

Procedure:

E. faecalis culture [ATCC 29216] grown overnight in brain heart infusion (BHI) broth were divided in four groups. Group-I: 2.5% NaOCl (n=10), Group-II: Curcuma longa extract (n=10),Group-III: Azadirachta indica extract(n=10), Group-IV: Aloe barbadensis miller (n=10). All four study irrigants are added to respective wells in agar plates. Bacterial inhibition zone around each well was noted.

Calculation of Zone Of Inhibition:

After 72 hours, the MHA plates were checked for zones of inhibition. The degree of susceptibility or resistance of the test organism to the antibacterial agent was shown by the zone of inhibition or clear zone. The zone edge was determined to be the point at which growth significantly decreased and completely inhibited. With the help of a ruler, the forty plates inhibition zones were all measured (mm).

Insilico Study:

Preparation of Ligands For Docking:

The chemical structure of phytocompounds from curcuma longa,Azadirachta indica and Aloe barbadensis miller were obtained from PubChem compound database in SDF format. By using online smiles translator all the compounds were converted in to PDB form.All the compounds were loaded to PyRx window and changed to pdbqt format to perform the docking studies.[6]

Preparation of Receptor

Three dimensional structure of *E. faecalis* target proteins DHFR, Glutamate racemase , Alanine racemase , DNA ligaseand Topoisomerase DNA gyrase Bwas retrieved from PDB database. To remove natural ligand, the receptor data were downloaded and opened using Discovery Studio. The non-polar hydrogen atoms were added before docking, followed by Gasteiger charger calculation using Autodock tools. The protein file was then saved in pdbqt format and ready to be used for docking.[7]

Docking

AutoDock Vina, a molecular docking program in PyRx Virtual screening too was used for docking. The PDBQT format file containing the protein atom coordinates, and helps to determine around the active site of the protein. Dock scores were reported in kcal/mol using the Lamarckian Genetic Algorithm (LGA). The source of the ranked clusters of compound

conformations was examined in the result.[8,9]

Statistical analysis:

Data obtained from the microbiological study will be tabulated and statistically analyzed using Analysis Of Variance tests at $P < 0.001$.

Results:

Zone of inhibition in Group 1 was 27.75 with 0.9 (standard deviation) SD and ranging within 27-29 mm; Group 2 was 28.5 with 0.57 SD and ranging 28-29mm, Group 3 was 23.25 with 0.5 SD ranging within 23-24mm, Group 4 was 25.25 with 0.5 SD ranging within 25-26mm. Mean zone of inhibition was observed to be greater in Group 2 followed by Group 1, Group 4 and Group 3 respectively. The zone of inhibition created by four medicaments was tabulated (Table 1). All the medicaments were efficient in inhibition of E.faecalis and maximum inhibition was observed in Group 2 (28.5 ±0.57) . (Figure 1, Graph 1)

The molecular docking of the compounds are explained in table 2,3,4.

Table 1: The zone of inhibition created by the four medicaments

N		Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Aloe Vera Extract	4	25.25	.500	.250	24.45	26.05
Neem Extract	4	23.25	.500	.250	22.45	24.05
Turmeric Extract	4	28.50	.577	.289	27.58	29.42
Sodium Hypochlorite	4	27.75	.957	.479	26.23	29.27
Total	16	26.19	2.228	.557	25.00	27.37

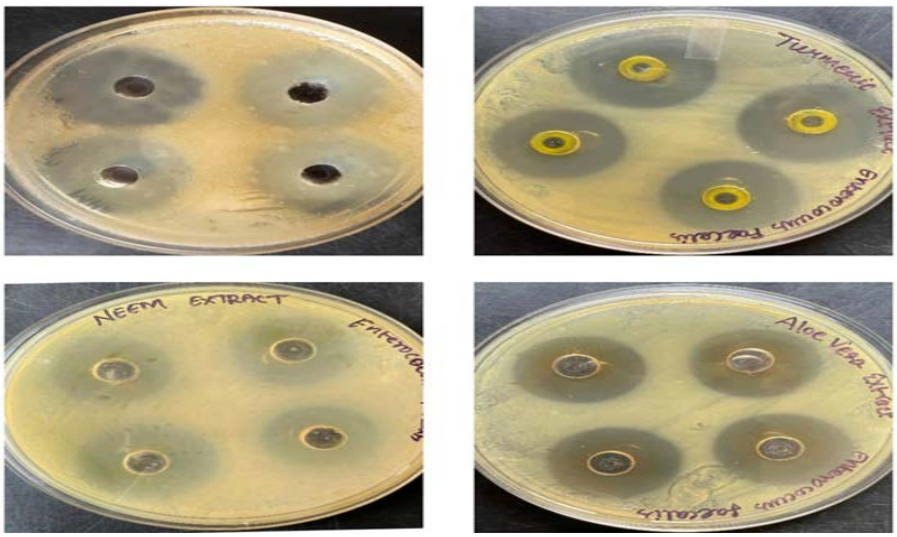
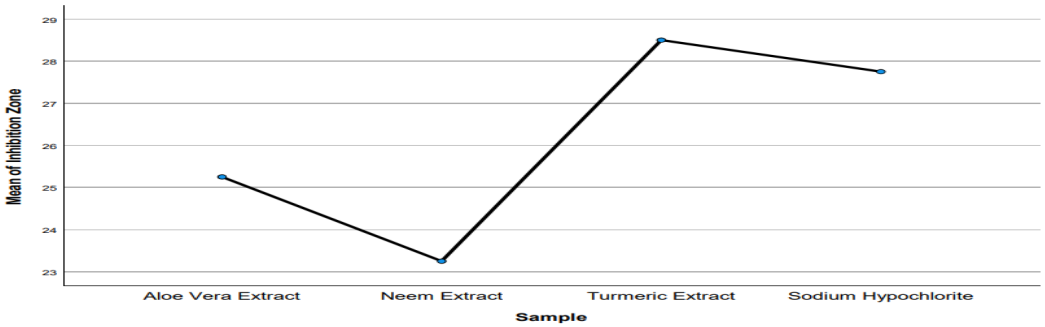


Figure 1: Zone of inhibition by the four medicaments a) GroupI:2.5%NaOCl b) Group II: Curcuma longa c) Group III: Azadirachta indica d) Group IV: Aloe barbadensis miller



Graph 1: Graphical representation of four medicaments

Table 1: Molecular docking with compounds from curcuma longa

S.No	Compound Name	Docking score	Hydrogen bond interaction
1	Ar-Turmerone	-6.3	
2	beta-Elemene	-6.1	
3	Curcumin	-5.6	
4	Curdione	-5.5	
5	Curzerene	-6.3	
6	Demethoxycurcumin	-6.5	ARG-144 MET-106 THR-103 ASN-26 ARG-30 ASN-167
7	Germacrone	-6.4	ILE-229
8	Isourecumenol	-6.3	
9	Zingiberene	-5.5	

Table 2: Molecular docking with compounds from Neem

S.No	Compound Name	Binding Energy Kcal/mol	Hydrogen Bond interaction
1	Mahmoodin	-6.8	
2	Margolonone	-8.7	LYS-34 SER-217
3	Meliantriol	-7.1	
4	Nimbidin	-7.2	
5	Nimbin	-6.4	
6	Nimbolide	-7.7	LYS-106 SER-217
7	Quercetin	-6.9	

Table 3: Molecular Docking With Compounds From Aloevera

S.No	Compound Name	Binding Energy kcal/mol	Hydrogen bond interaction
1	Ascorbic acid	-5.8	
2	Caffeic acid	-6.7	VAL-47 ASP-76 ARG-79
3	Catechol	-5.2	
4	Cinnamic acid	-6.2	
5	Ferulic acid	-6.7	
6	p coumaric acid	-7.4	ASP-76
7	Sinapic acid	-7	

Discussion:

The primary purpose of root canal therapy is to remove bacteria from the root canal and improve their mechanical properties. In the root canal, a vast variety of microorganisms exist, with Enterococcus faecalis being the most resistant. The capacity of E. *faecalis* to adhere to the dentin collagen and sustain within the tubule causes failure of an endodontically treated tooth. Intracanal medications such as calcium hydroxide and antibiotic pastes are used to prevent microbial development, with Ca (OH)2 having no possible effect on E. faecalis. So, the elimination of bacteria, their products and substrate enhance the success rate of endodontic therapy.

Herbal medications are becoming more admired due to their biocompatibility, anti-inflammatory and antibacterial characteristics, as well as their effectiveness as an intracanal medicament against *E. faecalis.*[10]

Chemomechanical root canal preparation entails both mechanical instrumentation and irrigation which is aimed primarily at removing microorganisms from the canal system. Because of their unique anatomic location, bacteria in the root-canal system are immune to the host's defences. As a result, endodontic infections can only be treated by a dentist using a mixture of chemical and mechanical methods. Chemomechanical preparation and interappointment medication are the two main aspects of endodontic treatment that concern with infection prevention. The mechanical action of tools, as well as the flow antibacterial irrigants are used to remove bacteria from the root canal.[11]

Endodontic infections are typically caused by E.faecalis, a gram-positive facultative anaerobe. It can battle with other microbes, infiltrate dentinal tubules, and withstand nutrient deprivation. Enterococcus faecalis is found in endocarditis, urinary tract infections, prostatitis, intra-abdominal infection, cellulitis, and wound infection, as well as concomitant bacteremia[12].

Herbal Extracts have numerous beneficial features, including antibacterial, anti-inflammatory, and antioxidant actions. They can be used to treat infections of the respiratory, digestive, and genitourinary systems, as well as skin diseases, and can be utilized as natural preservatives in cosmetics or pharmaceuticals. Possessing a good antimicrobial activity, Herbal Extracts can replace treatments with antibiotics and disinfection using antiseptics. Some Herbal extracts have been put to the test in the field of dentistry.

In the ligand protein docking calculations, based on the scoring parameters the best conformation for every ligand is chosen. Presences of hydrogen interaction in the docked complexes were used to stabilize the docked complex Docking studies revealed the interaction of the protein with the ligands, binding energy, type of interaction and amino acids involved in interactions. Table 1 give the binding energy of ligands with proteins respectively with inhibitors. Table 2 showed the results of docking studies of all target proteins with compounds. Based on the score we have selected best two compounds for each target proteins and analysed the hydrogen bond interaction. Table 3 showed that all the compounds showed the good interaction with target proteins. In this study, most of the complexes formed good number of hydrogen bond interaction with target proteins. Among the seven compounds selected from aloe vera , the compounds Caffeic acid and p coumaric acid showed the very strong interaction with all the target proteins with very good binding energy. And also, these complexes formed the so many hydrogen bonds interaction with all the target proteins. To test these phytotherapeutic agents and alter their content for patient acceptability, more in vivo research is needed.[13,14]

From the above done parameters it can be concluded that Curcuma longa (turmeric) revealed a zone of inhibition against E. faecalis with highest ligand binding energy in docking

followed by aloe vera and neem extract. As a result, these can be used to irrigate root canals.

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